

Ophiostoma Polonicum is a Species of *Ceratocystis sensu stricto*

CHRISTA VISSER¹, MICHAEL J. WINGFIELD¹, BRENDA D. WINGFIELD¹, and YUICHI YAMAOKA²

¹ Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300, South Africa

² Institute of Agriculture and Forestry, University of Tsukuba, Tennodai, Tsukuba, Ibaraki 305, Japan

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Summary

Ophiostoma polonicum was first isolated in Poland and described in 1939 by Siemaszko. The fungus is a serious pathogen of spruce and is vectored by the bark beetle *Ips typographus*. *Ophiostoma polonicum* was described as producing a *Leptographium* anamorph similar to that of *Ophiostoma penicillatum*. The fungus was later transferred to *Ceratocystis penicillata* but is currently treated as a species of *Ophiostoma*. Examination of the culture collected by Siemaszko and also from recent collections from spruce in Europe and Japan have shown the presence of a *Chalara* state. Cell polysaccharide analysis revealed the absence of rhamnose in the cells of *O. polonicum* which is more typical of *Ceratocystis* species. The fungus was also found to be sensitive to cycloheximide, which is characteristic of species of *Ceratocystis sensu stricto*. Comparisons of partial sequence data of the ribosomal DNA operon have also revealed that isolates of *O. polonicum* group together with *Ceratocystis laricicola*, which is a well defined species of *Ceratocystis sensu stricto*. We, therefore, conclude from this study that *O. polonicum* is a typical species of *Ceratocystis s.str.* and also that the species is very likely conspecific with *C. laricicola*.

Key words: rRNA – *Ophiostoma polonicum* – Phylogeny – *Ceratocystis* – *Ips typographus*

Introduction

The ophiostomatoid fungi include the genera *Ceratocystis* Ell. & Halst. and *Ophiostoma* Sydow and have been known since the early part of this Century. Most species in these genera have ascospores with elongate necks and they are generally considered to be vectored by insects (Wingfield et al. 1993). Numerous species of these fungi also include serious pathogens of trees of which *Ophiostoma ulmi* Buisson and *Ophiostoma novo-ulmi* Brasier (Brasier, 1993) and *Ceratocystis fagacearum* Bretz (Kile, 1993) are perhaps best known.

The taxonomy of *Ophiostoma* and *Ceratocystis* has had a confused history. These genera have been regarded as synonyms or as separate entities by various authors in the past. In the most recent and comprehensive treatment of the group, Upadhyay (1981) treated the genera as synonyms but included the new genus *Ceratocystiopsis* Upadhyay and Kendrick to accommodate species with falcate ascospores. Convincing evidence based on cell wall composition (Rosinski and Campana, 1964; Spencer and Go-

rin, 1971; Jewell, 1974; Weijman and De Hoog, 1975) has, however, been presented to support the notion that *Ceratocystis* and *Ophiostoma* are distinct. Species of *Ceratocystis* also produce conidia by ring wall building and have *Chalara* anamorphs (Minter et al. 1982) as opposed to apical wall building (*Graphium* Corda, *Leptographium* Lagerberg & Melin, *Sporothrix* Hektoen & Perkins, *Hyalorhinochlamydia* Upadhyay & Kendrick) in *Ophiostoma* anamorphs (Mouton et al. 1994). Unlike *Ceratocystis*, species of *Ophiostoma* are also resistant to high concentrations of the antibiotic cycloheximide (Harrington, 1981).

Recent studies at the molecular level have provided additional evidence for the fact that *Ceratocystis* and *Ophiostoma* are distinct. These studies have been based on analysis of the 18S ribosomal RNA gene and have shown that the genera are distantly related (Hausner et al. 1992, 1993a, 1993b, 1993c; Spatafora and Blackwell, 1994). Morphological similarities in teleomorph struc-

tures in these phylogenetically distinct groups have apparently developed as an adaptation to an insect-associated habitat.

Ophiostoma polonicum Siemaszko was first described in 1939 associated with the bark beetle (Coleoptera: Scolytidae) *Ips typographus* L. that infests spruce, *Picea abies* (L.) Karst. in Europe (Siemaszko, 1938). The fungus was described as having a *Leptographium* anamorph. Moreau (1952) later treated this fungus in the genus *Ceratocystis* as *Ceratocystis polonica*. No type material exists for *O. polonicum* but, based on the *Leptographium* state described by Siemaszko (1939), Upadhyay (1981) reduced this species to synonymy with *Ceratocystis penicillata* (Grosm.) Moreau (= *Ophiostoma penicillatum* (Grosm.) Siemaszko). Later, Solheim (1986) considered *O. penicillatum* and *O. polonicum* to be distinct species of varying pathogenicity (Solheim, 1991, 1993; Christiansen, 1985). Solheim (1986) was, however, unable to detect the *Leptographium* state of any other anamorph in *O. polonicum*.

Considerable confusion surrounds the generic placement of the fungus that Siemaszko (1939) named *O. polonicum*. During a recent survey of fungi associated with *Ips typographus japonicus* infesting *Picea jezoensis* (Sieb. et. Zucc.) Carr. in Hokkaido, Japan, we noted an apparent *Chalara* state in some isolates of a fungus otherwise resembling *O. polonicum*. This would indicate that the fungus is a *Ceratocystis* species as opposed to a species of *Ophiostoma*. The aim of this study was, therefore, to examine isolates of *O. polonicum* from Japan and Europe using a variety of techniques and, thus, to establish an appropriate generic placement for this fungus.

Material and Methods

Isolates examined. For the purposes of this study, we made morphological examinations of four isolates of a fungus matching the teleomorph characteristics of *O. polonicum* from Japan and Europe including the isolate collected by Siemaszko in Poland obtained from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands (CBS). Isolates from Europe were from *I. typographus*-infested *P. abies* and supplied by Dr. Solheim (Norwegian Forest Research Institute, Section of Forest Ecology, Division of Forest Pathology, N-1423 Ås-NLH, Norway). Those from Japan were collected by the last author from *I. typographus japonicus*-infested *P. jezoensis* in Hokkaido, Japan. All isolates included in this study are maintained in the culture collection of the second author, with representative isolates also available in other collections.

For the experimental part of this study, four isolates of *O. polonicum* were utilised. These include one from Japan (YCC118 [cmw 2274]) and three from Europe (ATCC 62335 [cmw 1164], CBS 133.38 [cmw 672], cmw 2443). The European isolates included two isolates supplied by H. Solheim as well as an isolate (cmw 672) collected by Siemaszko who originally described this fungus. Two isolates of *Ceratocystis laricicola* Redfern & Minter (cmw 1016 and cmw 1017) supplied by Dr. R. B. Redfern (Forestry Commission, Northern Research Station, Roslin, Midlothian EH25 9SY, UK) were also included given the fact that this fungus is very similar to *O. polonicum*, with the exception of the purported *Leptographium* state of the latter species.

Morphological examination of *O. polonicum* was done using light microscopy. Perithecia were abundant in numerous isolates including cmw 2274 from Japan and cmw 1164 from Europe and teleomorph characteristics could be compared with those described for this fungus by Siemaszko (1939). Cultures were also examined thoroughly for the presence of anamorph characters.

Cycloheximide tolerance and Cell polysaccharide analysis. All isolates of *O. polonicum* used in the experimental part of this study were tested for their ability to tolerate cycloheximide in culture. In addition, an isolate of *Ceratocystis fimbriata* Ell. & Halst. and *Ophiostoma piceae* Munch were included for comparative purposes. Five different concentrations (0.00, 0.05, 0.10, 0.50, 1.00 and 2.50%) of cycloheximide in 2% Malt extract agar (MEA; 20 g malt extract and 20 g agar/l dH₂O) were tested. Agar discs, 4.2 mm in diameter were cut from the actively growing margin of cultures and placed at the centres of three Petri dishes containing the different concentrations of cycloheximide for each isolate. Two diameter measurements were taken for each colony after incubation in the dark for six days. Averages of these measurements were calculated and this experiment was repeated once.

Isolates of *O. polonicum*, for the cell polysaccharide analysis, were grown in 15 g ME (malt extract) and 5 g Glucose/l dH₂O at 20°C for two weeks. Cell material was collected by centrifugation at 10 000 rpm for 10 min, washed twice with ddH₂O and lyophilised. The freeze-dried cell material was hydrolysed with 72% H₂SO₄ for 12 h at 4°C, followed by boiling for 2.5 h in 2 M H₂SO₄ and then neutralised with NaOH. Presence of cell saccharides was determined by column chromatography using a Technicon Auto Analyser System (Alsa Tech, Isando, Johannesburg, South Africa) as previously described by Van Biljon and Olivier, 1989.

Sequence comparisons. Cultures were grown on cellophane discs placed on 2% MEA and incubated at 20°C until the mycelium covered the disc. These cellophane discs were then transferred to sterile Petri dishes, lyophilised and stored at -20°C. Nucleic acid was extracted from the freeze-dried material using a modified Guanidinium Thiocyanate procedure of Chirgwin et al. (1979). A region within the ribosomal DNA operon, including the variable Internal Transcribed Spacer regions (ITS1 and ITS2) as well as the conserved 5.8S gene, were amplified using the Polymerase Chain Reaction (PCR), Saiki, 1988). Primers ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3') (100D/ml) were used for amplification (White et al. 1990). Reactions were performed in a Hybaid Omnigene Temperature Cycler (Hybaid, Middlesex, UK) for 35 cycles using *Taq* DNA polymerase (Promega Corporation, Madison, U.S.A.) with the Magnesium free 10x Buffer supplied by the manufacturer. The final buffer composition was 6.25 mM MgCl₂, 1.6 mM of each dNTP and 360 pmol of each primer. An initial 5 min denaturation step at 96°C was performed, followed by 35 cycles of 92°C for 60 seconds (denaturation), 55°C for 30 seconds (annealing) and 72°C for 60 seconds (extension) with a final elongation step of 5 min at 72°C. The PCR products were visualised on a 1% (w/v) agarose gel to assess the amplification. The amplified DNA products were purified using the Magic PCR Preps (Promega Corporation, Madison, U.S.A.) and sequenced with the *fmol* Sequencing System (Promega Corporation, Madison, U.S.A.). Primers ITS4, ITS1, CS2 (5'CAATGTGC-GTTCAAAGATTTCG3') and CS3 (5'CGAATCTTTGAACG-CACATTG3') [constructed by B. D. Wingfield, University of the Orange Free State] were used to determine the DNA sequence in both directions. The sequence data were visually aligned and the phylogenetic relationships determined using PAUP (Phylogenetic Analysis Using Parsimony) (Swofford et al., 1993). Both the branch and bound and the heuristic options were used in the PAUP analysis. DNA sequence of *C. fimbriata*, the type species of

Ceratocystis s.str. was included in this study for comparative purposes. *Neurospora crassa* sequence (Chambers, 1986) was used as an outgroup.

Results

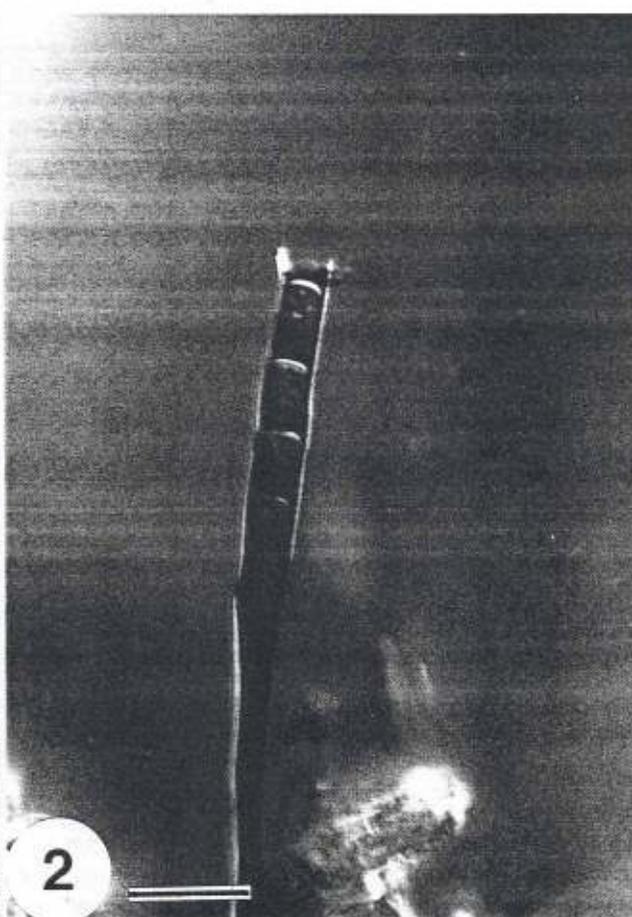
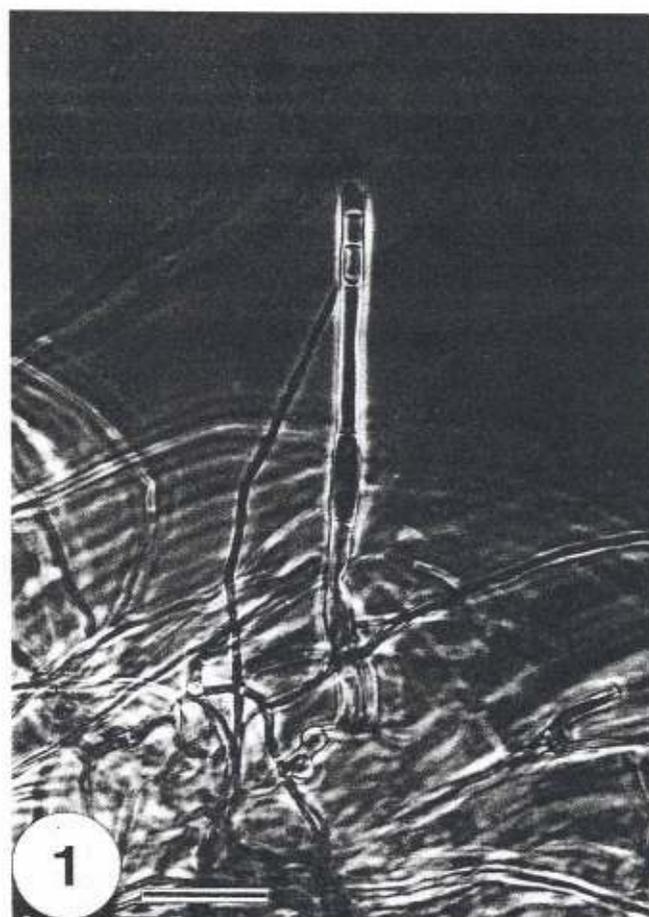
Morphological comparisons. Cultural characteristics of the four *O. polonicum* isolates were similar and differed markedly from those previously described for *O. penicillatum*. The former fungus has a light grey colour with profuse aerial mycelium as opposed to the dark green-grey colonies of *O. penicillatum*. Isolate cmw 672 collected by Siemaszko (1939) produced no fruiting structures. Other isolates of the fungus produced distinct ophiostomatoid ascomata with single-celled, reniform, sheathed, two guttulate ascospores identical to those described by Siemaszko (1939). Although these structures were extremely rare, conidiophores (Fig. 1) and conidia (Fig. 2) typical of *Chalara* species were occasionally found associated with the bases of the ascomata in many isolates.

Cycloheximide tolerance and cell wall polysaccharides. All four isolates of *O. polonicum* tested for tolerance to cycloheximide, proved to be sensitive to all concentrations

of this antibiotic, even to as low as 0.05 g/l of cycloheximide (Table 1). In this characteristic they were similar to

Table 1. Average growth in mm of *Ophiostoma polonicum* isolates on various concentrations of cycloheximide. *Ophiostoma piceae* and *Ceratocystis fimbriata* were included for comparative purposes

Fungus	% Cycloheximide (g/l)					
	0.00	0.05	0.10	0.50	1.00	2.50
<i>Ophiostoma polonicum</i>						
cmw 1164 (Sweden)	44.8	-	-	-	-	-
cmw 2443 (Sweden)	21.2	-	-	-	-	-
cmw 2274 (Japan)	19.5	-	-	-	-	-
cmw 672 (Poland)	34.1	-	-	-	-	-
<i>Ceratocystis fimbriata</i>						
cmw 2220	33.0	-	-	-	-	-
<i>Ophiostoma piceae</i>						
cmw 153	48.2	40.9	41.0	38.3	35.0	37.1



Figs. 1-2. Conidiophore and conidia of *O. polonicum*. Fig 1. Light micrograph showing the typical *Chalara* state observed for *O. polonicum* (Bar: 15 mm = 20 μ) and Fig 2. showing the conidia produced by the conidiophore of *O. polonicum* (Bar: 15 mm = 10 μ).

		10	20	30	40	50
<i>N. crassa</i>	TCATTACAGA	GTTGCAAAAC	TCCCACAAAC	CATCGCGAAT	CTTACCCGTA	
cmw 2220	-----	C.GATTCTTG	.AGTGAG.TG	A..G.T.TT	TGGTGGTAGG	
cmw 672T.-	AG.NNNNGGA	-.T.TT....	...AT-.TGA	ACATA..TNN	
cmw 1017T.G	AG.TTTT--A	A.T.TT....	...AT-.TGA	ACATA..T.T	
cmw 2443T.-	AG.TTTT--A	A.T.TT....	...AT-.TGA	ACATA..T.T	
cmw 1016T.-	AG.TTTT--A	A.T.TT....	...AT-.TGA	ACATA..T.T	
cmw 2274T.-	AG.NNNNGGA	-.T.TT....	...AT-.TGA	ACATA..TNN	
		60	70	80	90	100
<i>N. crassa</i>	CGGTTGCCTC	GGCGCTGGCG	GTCC-GGA--	-AAGG-CCIT	CGGGCCTCCC	
cmw 2220	GCCC.T.TGA	A.G.-CAC..	C.G.CA.CAG	T.TA.T.--.	..CCA..GT-	
cmw 672	.CCA-.TG.	TTT.GCA.GT	C.TGG-T.AA	AC.A.T-.C.	GCC.GTAGTA	
cmw 1017	TTTA-.TG.	TTT.GCA.GT	C.TGG-T.AA	AC.A.T-.C.	GCC.GTAGTA	
cmw 2443	NTTA-.TG.	TTT.GCA.GT	C.TGG-T.AA	AC.A.T-.C.	GCC.GTAGTA	
cmw 1016	TTTA-.TG.	TTT.GCA.GT	C.TGG-T.AA	AC.A.T-.C.	GTC.GTAGTA	
cmw 2274	.CCA-.TG.	TTT.GCA.GT	C.TGG-T.AA	AC.A.T-.C.	GCC.GTAGTA	
		110	120	130	140	150
<i>N. crassa</i>	T-----AAA	CTCTTGA-TA	TPTATGTCCT	TCTGAGTAAA	CTTTTAAATG	
cmw 2220TCCAGAT	.T.TTCATTG	..GAGTGGCA	
cmw 672	.TTAGAA...T.A..	...C.AGAGA	AT.T.TAATC	A..GCTG.GC	
cmw 1017	.TTAGAA...T.A..	...C.AGAGA	AT.T.TAATC	A..GCTG.G-	
cmw 2443	.TTACAA...T.A..	...C.AGAGA	AT.T.T.ITC	A..GCTG.G-	
cmw 1016	.TTAGAA...T.A..	...C.AGAGA	AT.T.TAATC	A..GCTG.G-	
cmw 2274	.TTAGAA...T.A..	...C.AGAGA	AT.T.TAATC	A..GCTG.GC	
		160	170	180	190	200
<i>N. crassa</i>	GATCCTCGGG	TCTCCGCTCG	CGGCTGCCCG	COGGAGTGCC	GAAACAAGTC	
cmw 2220	T.A.--TA--	-----	-----	-----	T...A...T	
cmw 672	TGG.A.TAA-	-----	-----	-----	.A T..TA.GC.T	
cmw 1017	TGG.A.TAA-	-----	-----	-----	.A T..TA.G-.T	
cmw 2443	TGG.A.TAA-	-----	-----	-----	.A T..TA.G-.T	
cmw 1016	TGG.A.TAA-	-----	-----	-----	.A T..TA.G-.T	
cmw 2274	TGG.A.TAA-	-----	-----	-----	.A T..TA.GC.T	
		210	220	230	240	250
<i>N. crassa</i>	AAAACCTTCA	ACAACGGATC	TCTTGGTCT	GGCATCGATG	AAGAACGCAG	
cmw 2220C...	A.....	
cmw 672C...	A.....	
cmw 1017C...	A.....	
cmw 2443C...	A.....	
cmw 1016T....C...	A.....	
cmw 2274C...	A.....	
		260	270	280	290	300
<i>N. crassa</i>	CGAAATGCGA	TA-GGTAATG	TGAATTGCAG	AATTCAGTGA	ATCATCGAAT	
cmw 2220A.-.....	
cmw 672C..C....-.....	-.....	
cmw 1017C-.....NN.	
cmw 2443C-.....	
cmw 1016C-.....	
cmw 2274C..C....-.....	-.....	

Fig. 3. Alignment of the DNA sequences obtained for the ITS1, ITS2 regions and the 5.8S rRNA gene for 3 isolates of *O. polonicum* (cmw 672, cmw 2274 & cmw 2443), 2 isolates of *C. laricicola* (cmw 1016 & cmw 1017), one isolate of *C. fimbriata* (cmw 2220) and *N. crassa* which was used as an outgroup. N indicates unknown bases; a dot indicates bases identical to the corresponding base in *N. crassa* and dashes represent deletions in the sequence.

C. fimbriata (type species of *Ceratocystis s.str.*), but differed markedly from *O. piliferum* (typical species of *Ophiostoma*). Rhamnose was found to be absent in all the isolates of *O. polonicum* tested for cell polysaccharides.

Sequence comparisons. A single DNA fragment of about 550 bp was observed using gel electrophoresis for all isolates amplified. For each isolate amplified, a total of

about 500 bp were sequenced and read (Fig. 3). The DNA sequences obtained were visually aligned and edited using MacClade 3.0 (Fig. 3) (Maddison & Maddison, 1992). The sequence of *Neurospora crassa* which was included in this study for comparative purposes was obtained from Chambers et al. (1986). The bootstrap analysis for each option resulted in the same tree configuration and a strict

	310	320	330	340	350
<i>N. crassa</i>	CTTTGAACGC	ACATTGCGC-	TCGCCAGTAT	TCTGGCGAGC	ATGCCTGTTC
cmw 2220	..N.....C.C	.G.N-.....C.AG..-
cmw 672	..C...--..GC.C	.G-.....	...-C.AGCA
cmw 1017	..N...N...N.C	.G-.....C.AGCA	C.....C.
cmw 2443GC..	.G-.....C-AG-A	N.....C.
cmw 1016C	.G-.....C.AGCAC.
cmw 2274	..C...--..GC.C	.G-.....	...-C.AGCA
	360	370	380	390	400
<i>N. crassa</i>	--GAGCGTCA	TTTCAACCAT	CAAGCTCTGC	TT--GCG-TT	GGGGATCCGC
cmw 2220	C.....C.AC.GA..C.	..TGNNC...	..C.T.GGAG
cmw 672	C.-.....C.AC.G.T....	..A.GA..CG
cmw 1017	G.-.....N...C.AC.GN.....	..A.GA..CG
cmw 2443	G.-.....C.AC.G.T....	..A.GN..CG
cmw 1016	G.-.....C.AC.G-T....	..A.GA..AG
cmw 2274	C.-.....C.AC.G.T....	..A.GA..CG
	410	420	430	440	450
<i>N. crassa</i>	G-GCTGTCCG	CTCAAATCA	GTGGCGGGCT	CGTCAGTCAC	ACCGAGCGTG
cmw 2220	.TC....T.T	.C.CTG.A..	.GCCGCC.AA	ATGT.TC-GG	CTGTTATACT
cmw 672	CA--.C.T--	-----G--.G	.GCCGCC.AA	ATG..TC-GG	CTGTT-GAAT
cmw 1017	CA--.C.T--	-----G--.G	.-CCGCC.AA	ATG..TC-GG	CTGTTCGAAT
cmw 2443	CA--.C.T--	-----G--.G	.-CCGCC.AA	ATG..TC-GG	CTGTT-GAAT
cmw 1016	-.....	-----	-----	-----	-----
cmw 2274	CA--.C.T--	-----G--.G	.GCCGCC.AA	ATG..TC-GG	CTGTT-GAAT
	460	470	480	490	500
<i>N. crassa</i>	GTGACTCTAC	ATCGCTATGG	TCGTGCGGCG	GG-----	-----
cmw 2220	T-.C.AACT.	CC.TG.G.A.	.ATAAAATTT	CTAATTTTTT.	ACACTTTTGAA
cmw 672	T..CAG..T.	CC-TG.G.A.	.AA.ATT-A-	TTTTTTT...	ACGCTTT.GA
cmw 1017	T..CAG..T.	CC-TG.G.A.	.AA.ATTTA-	TTTTTTT...	AGCGCTTTGA
cmw 2443	T..CAG..T.	CC-TG.G.A.	.AA.ATTTA-	TTTTT.....	ACGCTTT.GA
cmw 1016	-----T.	CC-TG.G.A.	.AA.ATTTA-	TTTTTTT...	ACGCTTT.GA
cmw 2274	T..CAG..T.	CC-TG.G.A.	.AA.ATT-A-	TTTTTTT...	ACGCTTT.GA
	510	520	530	540	550
<i>N. crassa</i>	--TTC-TT--	-----GC	CG-TAAAACC	CCCC-----	-----
cmw 2220	.G.....GT	G.TAACAC..	..C....-	AA..CCCTCA	ACTTTTGTGTG
cmw 672	AAC.....TT	A.CAACATCG	.CG.....A..TCA	ATTTT.....
cmw 1017	AAC.....TT	A.CAACATCG	.CG.....TCA	ATTTT.....
cmw 2443	AAC.....T.	..CA.CATCG	.CG.....TCA	ATTAT.....
cmw 1016	AAC.....TT	A.CAACATCG	.CG.....TCA	ATTTT.....
cmw 2274	AAC.....TT	A.CAACATCG	.CG.....A..TCA	ATTTT.....
	560				
<i>N. crassa</i>	-A-TTTC-TA	AGGTTGACC			
cmw 2220	A.C....AC.			
cmw 672	.-.....G.			
cmw 1017	.-.....A.	.AC.....			
cmw 2443-GA.			
cmw 1016	.-.....NA.			
cmw 2274	.-.....G.			

consensus tree was constructed (Fig. 4). The *g*₁ statistics determined from the bootstrap trees obtained for the branch and bound and the heuristic searches were -1.89 and -1.74, respectively. The *O. polonicum* isolates formed a cluster with *C. fimbriata* with a confidence interval of a 100%, confirming other evidence that these isolates are most likely species of *Ceratocystis*. The isolate from Japan

grouped together with Siemaszko's isolate with a confidence interval of 100% and these two clustered with the two *C. laricicola* isolates with 74% confidence (Fig. 4). The Norwegian isolate of *O. polonicum* was less closely related to the above-mentioned isolates, although more closely related to these than to *C. fimbriata*.

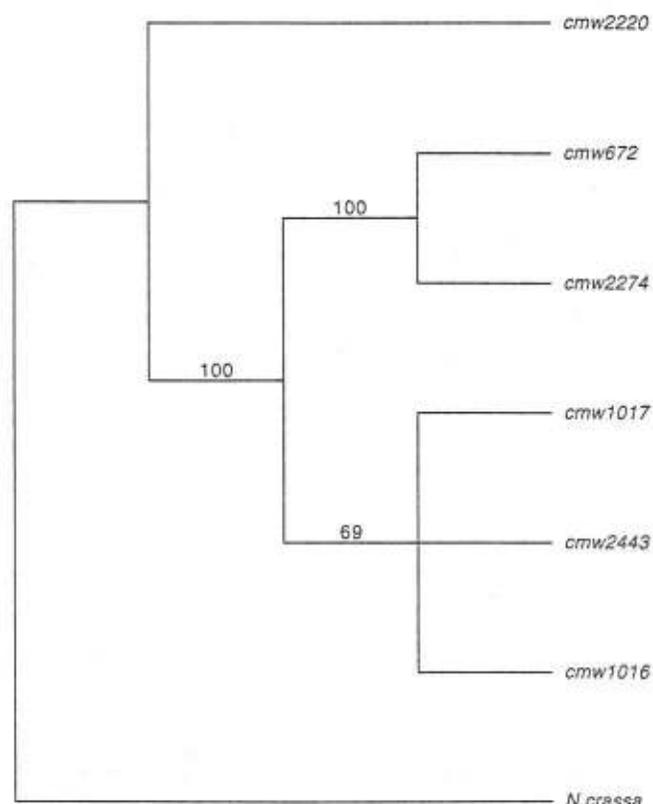


Fig. 4. Consensus tree generated from PAUP analysis depicting the phylogenetic relationships between *C. laricicola* (cmw 1016 & cmw 1017), *O. polonicum* (cmw 672, cmw 2274 & cmw 2443) and *C. fimbriata* (cmw 2220). *Neurospora crassa* was used as an outgroup. Bootstrap values are indicated at the branch points.

Discussion

Results of this study show that the fungus that was described as *O. polonicum* is a typical species of *Ceratocystis s.str.* The most definitive characteristics that lead us to this conclusion are the presence of a *Chalara* anamorph (Minter et al. 1982; De Hoog and Scheffer, 1984), sensitivity to cycloheximide (Harrington, 1981) and the absence of rhamnose in the cell walls (Rosinski and Campana, 1964; Weijman and De Hoog, 1975 and Spencer and Gorin, 1971). This fungus can easily be separated from *O. penicillatum*, which has a *Leptographium* anamorph and is tolerant to cycloheximide. We suggest, therefore, that the name *Ceratocystis polonica* should be used for this fungus.

In addition to the above characteristics, based on rDNA sequence analyses, it was possible to confirm that isolates of *C. polonica* form a distinct group of fungi, closely related to *C. fimbriata* and distinct from *N. crassa*. The fact that two isolates of *C. laricicola*, a very well defined species of *Ceratocystis*, grouped together with *C. polonica* also supports the new generic placement of *O. polonicum* in *Ceratocystis s.str.* These data also strongly suggest that

C. laricicola and *C. polonica* are the same species although detailed morphological comparisons are required before a synonymy can be presented.

Past confusion pertaining to the generic placement of *C. polonica* is relatively easy to explain. This fungus is one of numerous ophiostomatoid fungi including *O. penicillatum*, that is carried by *Ips typographus* infesting spruce in Europe (Solheim, 1986). It, thus, occurs in close association with the latter fungus and the two are often isolated as mixed cultures (M. I. Wingfield, unpublished). It is our view that when this fungus was first collected, Siemaszko was dealing with a mixed culture of the two fungi and that he incorrectly identified the *Leptographium* state as being connected with ascomata of *C. polonica*. Upadhyay (1981) did not have access to type material of this fungus and evidently chose to synonymise it with *O. penicillatum* based on reported presence of a *Leptographium* state by Siemaszko (1938). Solheim (1986) later recognised that *C. polonica* was distinct from *O. penicillatum* but did not recognise that it might belong in *Ceratocystis* rather than in *Ophiostoma*.

Species of *Ophiostoma* and *Ceratocystis* represent excellent examples of distinct taxa that have evolved convergently in adaptation to insect dispersal (Hausner et al. 1993c; M. J. Wingfield et al. 1994; Spatafora and Blackwell, 1994; B. D. Wingfield et al. 1994). The discovery that the fungus previously known as *O. polonicum*, is in fact a species of *Ceratocystis s.str.*, makes it the first example of these two phylogenetically distinct groups occurring associated with the same insect in a specialised niche.

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